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Animal behaviour

Ultrasonic signalling by a Bornean frog

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Among anuran amphibians, only two species, *Odorrana tormota* and *Huia cavitympanum*, are known to possess recessed tympanic membranes. *Odorrana tormota* is the first non-mammalian vertebrate demonstrated to communicate with ultrasonic frequencies (above 20 kHz), and the frogs' sunken tympana are hypothesized to play a key role in their high-frequency hearing sensitivity. Here we present the first data on the vocalizations of *H. cavitympanum*. We found that this species emits extraordinarily high-frequency calls, a portion of which are comprised entirely of ultrasound. This represents the first documentation of an anuran species producing purely ultrasonic signals. In addition, the vocal repertoire of *H. cavitympanum* is highly variable in frequency modulation pattern and spectral composition. The frogs' use of vocal signals with a wide range of dominant frequencies may be a strategy to maximize acoustic energy transmission to both nearby and distant receivers. The convergence of these species' call characteristics should stimulate additional, phylogenetically based studies of other lower vertebrates to provide new insight into the mechanistic and evolutionary foundations of high-frequency hearing in all vertebrate forms.

Keywords: *Odorrana tormota*; *Huia cavitympanum*; ultrasonic communication; ultrasound; convergence

1. INTRODUCTION

Among the vocal vertebrates, anuran amphibians have long been considered the champions of acoustic simplicity. The majority of frogs and toads produce repetitive, highly stereotyped calls that they use to guide a limited number of behaviours (Wilczynski & Ryan 1988, but see Narins *et al.* 2000 and Christensen-Dalsgaard *et al.* 2002 for exceptions). Generally, call notes show little spectral, temporal or amplitude variation and contain frequencies between approximately 100 Hz and 5–6 kHz (Narins & Capranica 1977; Brenowitz *et al.* 1984; Schwartz & Wells 1986; Glaw & Vences 1994). To help ensure that an appropriate behavioural response is evoked during acoustic communication, the anuran auditory system is often tuned to salient spectral and/or temporal features of the conspecific call (Frishkopf *et al.* 1968; Capranica & Moffat 1975; Capranica & Rose 1983). The elegant coevolution of this relatively straightforward acoustic system has made anurans an extremely valuable neuroethological model for the study of acoustic communication.

Recent behavioural and neurophysiological research suggests that the tenet of acoustic simplicity may not hold true for all anurans. Among the most striking examples is *Odorrana tormota* (previously *Amolops tormotus*), a frog found in two provinces of central China (Zhou & Adler 1993). This species has unusually high-pitched calls containing substantial energy in the ultrasonic frequency range (above 20 kHz), and its hearing extends from less than or equal to 1 kHz to approximately 35 kHz (Narins *et al.* 2004; Feng *et al.* 2006), dramatically exceeding previously reported upper limits of anuran frequency sensitivity (e.g. 8 kHz, Loftus-Hills & Johnstone 1970; 5 kHz, Fay 1988). Playback experiments demonstrate that the ultrasonic elements are behaviourally relevant and may be used to avoid masking by broadband but predominately low-frequency stream noise in their habitat (Narins *et al.* 2004; Feng *et al.* 2006). *Odorrana tormota* is the first non-mammalian vertebrate shown to communicate with ultrasound, and its use of this high-frequency channel for intraspecific acoustic communication challenges current understanding of frog sound production and reception mechanisms. It is unclear, however, whether ultrasonic communication is limited to *O. tormota*, or is more widespread among anurans.

The auditory periphery of *O. tormota* is characterized by a highly unusual morphological feature: the tympanic membranes are embedded in the skull at the end of canals, similar to those of mammals (Feng *et al.* 2006). These recessed tympana are hypothesized to play a critical role in the reception of ultrasound by facilitating transmission of high-frequency sound waves through the middle ear. Currently, only one other anuran species, *Huia cavitympanum*, is known to have recessed tympanic membranes. *Odorrana tormota* and *H. cavitympanum* are both southeast Asian species in the family Ranidae, yet they do not overlap in geographical distribution and are unrelated at the generic level (Cai *et al.* 2007; Stuart 2007). The habitats in which the frogs are found, however, are remarkably similar; males of both species call in close proximity to rushing streams that produce substantial broadband background noise. Given the similarity of the species' acoustic environment and peripheral auditory morphology, we predicted that they may have converged on the use of ultrasound for intraspecific communication. For this reason, we went to Borneo to record the calls of *H. cavitympanum* in their natural habitat using ultrasonic detection and recording equipment to examine the role of ultrasound in their vocal repertoire.

2. MATERIAL AND METHODS

Spontaneous vocalizations of males of *H. cavitympanum* were recorded from 28 June to 2 July 2007 along the banks of the Nyipa River adjacent to Camp 1, Gunung Mulu National Park, Sarawak, Malaysia (04° 03′ N; 114° 51′ E). Camp 1 is located at 170 m elevation in a multistoried mixed lowland dipterocarp forest. Ambient temperature and humidity were measured nightly with a digital thermohygrometer (Traceable Humidity/Temperature Pen, Fisher Scientific) to the nearest 0.1°C, and ranged between 23.8–26.8°C and 88–97%, respectively. Sound recordings were made between 18.00 and 21.00 using a portable digital recorder (Sound Devices 722) and a wideband microphone and preamplifier (G.R.A.S. 40 BE and 26 CB, respectively). The frequency response

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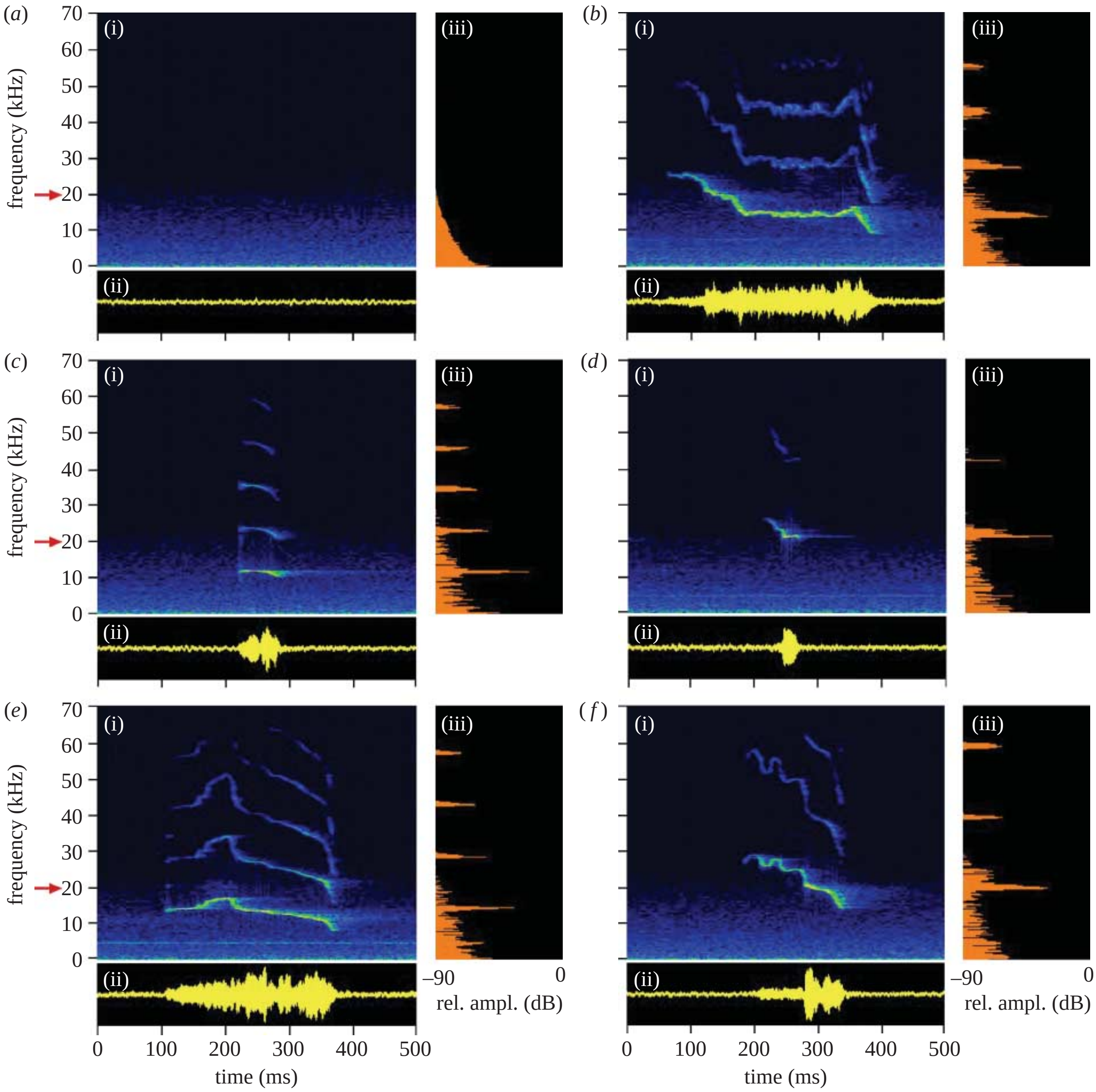

Figure 1. (i) Sound spectrograms, (ii) waveforms and (iii) instantaneous amplitude spectra (taken from the approximate centre of the recording) of (*a*) the background noise and (*b–f*) five representative calls given by a male frog over a 7 min period, demonstrating the diversity of its vocal repertoire. Arrows denote the boundary between audible and ultrasonic frequencies. Temperature during recordings is 26°C.

of the recording system was flat from 0.004 to 100 kHz±1.5 dB. The frogs called from the ground on steeply graded slopes approximately 5–10 m from the water's edge, and recording distance varied between 1 and 2 m depending on accessibility of the calling location. Calls were digitized at either 96 or 192 kHz sampling rate. Several vocalization bouts were filmed with a high-resolution digital video camera (Sony HDR-HC3 Handycam 4MP), which facilitated the identification of individual male's calls.

Animal vocalizations were saved as wave files on a removable Compact Flash card (4 GB Kingston Technologies Ultimate 133X) and copied nightly to the digital recorder's internal hard disk (40 GB). Upon return from the field, files were transferred to the computer via FireWire and analysed (FFT, 1024) and displayed using Selena, a custom-designed program (S. Andrzheevski, St Petersburg).

3. RESULTS

We recorded and analysed the calls of 5–10 *H. cavitympanum* males. Three frogs were positively identified and their calls were used to examine individual vocal signatures. Background noise at the calling sites was primarily produced by the rushing water of the adjacent river; at a distance of approximately 5 m from the bank, this noise was broadband with a peak of approximately 65 dB SPL near 100 Hz, decaying by 12 and 41 dB at 2 and 28 kHz, respectively (figure 1*a*).

Call features were highly variable both within and among individuals. The majority of calls showed some degree of downward frequency modulation, although the timing, slope, bandwidth and degree of warble of this component were variable (figure 1*b–f*). A subset of calls had a carrier of constant frequency, although most were preceded and/or followed by a short FM component (figure 1*c*). In addition, dominant frequencies (DF) of the call notes of the identified individuals varied over an average of 15 477±1545 Hz ($n=3$). Call DFs of the individual males fell into bimodal distributions consisting of low-frequency (11 673±205 Hz (mean±s.e.m. from three frogs), range 11 521–11 906 Hz) and high-frequency (20 258±599 Hz, range 19 730–20 908 Hz) clusters.

Within the high-frequency group, the energy of 14% of the calls fell completely within the ultrasonic range (figure 1*d*). The spectral energy of an additional 38% was entirely above 18 kHz and inaudible to the authors of this paper.

Calls were either produced singly or in series consisting of two to six notes. Within a call series, the first notes were significantly longer than the subsequent notes (unequal var, $t=9.7$, $n=68$, 179, $p<0.001$) and had a lower DF (unequal var, $t=-12.8$, $n=64$, 169, $p<0.001$). The large majority (89%) of purely ultrasonic calls were identified as being produced as the second through the sixth note of these call sets. Frequently, the temporal and spectral features of the second to sixth call notes within a series were somewhat less variable than singly emitted calls; however, these features still varied markedly between separate call series.

4. DISCUSSION

The vocal repertoire of *H. cavitympanum* is rich, varied and, unexpectedly, includes exclusively ultrasonic vocalizations, a feature previously undocumented in anurans. In contrast, the DF of the calls of the other 'ultrasonic' frog, *O. tormota*, are consistently within the audible range, 5–9 kHz (Feng *et al.* 2002; Narins *et al.* 2004). To our knowledge, *H. cavitympanum* and the blue-throated hummingbird (*Lapornis clemenciae*; Pytte *et al.* 2004) are the only non-mammalian vertebrates shown to produce structurally independent ultrasonic signals (i.e. not harmonic elements of audible sounds).

The use of an ultrasonic communication channel represents an intriguing trade-off of potential benefits and costs for signal transmission. Like *O. tormota*, *H. cavitympanum* may gain from an increased signal-to-noise ratio of its high-frequency call components in the midst of low-frequency ambient stream noise. In addition, producing high-frequency sounds reduces the mismatch between call wavelength and size of the radiating structure (i.e. vocal sac). This increases the coupling efficiency of metabolic to acoustic energy, allowing the frog to produce louder calls with less effort (Ryan 1988). Such an energetic advantage may explain why the majority of ultrasonic signals were given in the latter part of a call series, when the frog is presumably tiring from the vocal effort and benefits the most from increased metabolic efficiency. On the other hand, a potential cost of ultrasonic vocalization is increased signal attenuation and degradation, which may substantially reduce transmission distance (Marten *et al.* 1977). The extraordinary range of DFs used in *H. cavitympanum* calls may represent a bet-hedging strategy to maximize acoustic energy reaching receivers over a variety of distances.

How intended receivers of *H. cavitympanum* calls, such as gravid females or competitive males, detect their conspecific signals, presents a fertile avenue for further research. Anurans are unique among vertebrates in having two inner ear organs specialized for the reception of airborne sound: the amphibian papilla (AP) and basilar papilla (BP). Frequency sensitivity is divided between the two organs, and the BP is responsible for transducing high-frequency sounds. Hitherto, the highest frequency found to be detected by a frog's BP is 8.2 kHz (Loftus-Hills & Johnstone 1970); the DF of more than 97% of *H. cavitympanum* calls is above this value. It remains to be determined whether this species has dramatically extended the upper-frequency sensitivity of its BP, or whether another undescribed mechanism is at work. Peripheral to the inner ear, the role of recessed tympana in anuran ultrasound detection remains to be tested experimentally. The convergence of this morphological feature in two distantly related frog species that specialize in high-frequency communication suggests that future, phylogenetically based research on the relatively simple auditory systems of lower vertebrates could inform us of fundamental evolutionary, ecological and physiological attributes that confer high-frequency hearing sensitivity in all forms of vertebrate life.

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